TIME-PLACE LEARNING: CAN RATS TIME BOTH PERIODS OF FOOD AVAILABILITY AND NON-AVAILABILITY?

by © Pamela Chaulk

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Abstract

Animals learn and use information about the times and locations of food availability. The goal of the current study was to determine how rats time spatially and temporally predictable periods during which no food is available. The first experiment consisted of a daily time-place learning (TPL) task, with sessions conducted on a plus maze at three times each day. The rats learned to avoid the lever that did not provide reinforcement and had longer latencies to first lever press at non-reinforced times of day. Skip session probes showed that the rats tended to use a circadian timing strategy to solve the task. The second experiment consisted of an interval TPL task conducted in an operant box with four levers. Depending on group assignment, the rats experienced breaks between lever activation of either 0 s, 30 s, or 60 s. All rats learned the task, showing both anticipation and anticipation of depletion of a lever's active period. Despite having learned the task, there was no evidence that the rats timed the break periods. Probe tests revealed that the rats used an interval timing strategy to solve the task. Breaks in reinforcement did not prevent the rats in either experiment from learning the respective TPL tasks.

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Time-place learning: Can rats time both periods of food availability and non-availability?

Time-place learning (TPL) refers to the ability of animals to associate particular events with the location and time in which they occur (Thorpe, Bates, & Wilkie, 2003). The two types of TPL that have been the most extensively studied are daily and interval TPL. In daily TPL, animals learn that a particular significant event (e.g., food, predator) occurs in the same place at the same time each day (Carr & Wilkie, 1997b). Gallistel (1990) argued that an animal's memory is organized such that a code is created that represents a triad of relevant information; that is, the nature of the event, and the time and location at which it occurred. Future behaviour is guided by these memory codes, so as to allow expedient access to biological necessities, or to ensure avoidance of predation (Gallistel, 1990). In interval TPL, animals learn that the location of a significant event depends on the time that has passed since a previous event. For example, rats may be trained that food is available in Location A for 5 mins, Location B for the next 5 mins, and Location C for the final 5 mins. In interval TPL, the time period of interest is typically in the seconds to hours range (Carr & Wilkie, 1997b). Both daily and interval TPL research have consistently shown that animals are able to learn and use information about the times and locations in which reinforcement is available (e.g., Biebach, Gordijn, & Krebs, 1989; Reebs, 1999; Saksida & Wilkie, 1994). The current project was designed to determine whether rats also time periods of non-availability of food. In Experiment 1, rats were trained on a daily TPL task during which food was never available in one of three daily sessions. In Experiment 2, rats were trained on an interval TPL task during which there were non-reinforced periods interspersed in the standard interval TPL task.

Experiment 1: Daily Time-Place Learning

Events such as the presence of mates, food, and predators, are of particular importance to animals. These are often circadian based events and therefore vary depending on the time of day (Carr & Wilkie, 1997b; Reebs, 1996; Silver & Bittman, 1984; Thorpe & Wilkie, 2007). It is advantageous for animals to learn to predict when and where these events occur so that they may modify their behaviour based on these regularities. Research has shown that many species learn daily spatiotemporal variability (e.g., Reebs, 1999; Saksida & Wilkie, 1994; Schatz, Beugnon, & Lachaud, 1994; Tello-Ramos, Hurly, Higgott, & Healy, 2015). To study this ability in the laboratory, researchers often use daily TPL tasks in which animals are rewarded for going to one place at one time of day and to another place at a different time of day. One of the earliest laboratory studies of daily TPL was conducted by Biebach and colleagues (1989) in which garden warblers learned that each of four compartments provided grain for a specific 3 hr period each day. The birds learned to go to each compartment at the correct time of day, anticipated when each compartment would provide food as indicated by their tendency to enter a compartment just prior to when it started providing food, and continued to go to the correct compartment at the correct times during test sessions in which no food was given. Subsequent research has found evidence of daily TPL in a multitude of species including: fish (Reebs, 1999), ants (Schatz et al., 1994), hummingbirds (Tello-Ramos et al., 2015), honeybees (Wahl, 1932, as cited in Reebs, 1993), pigeons (Saksida & Wilkie, 1994), and rodents (Carr & Wilkie, 1997; Deibel & Thorpe, 2013). For example, Inanga fish learned that food was consistently placed in one side of their tank in the morning and in the other side of their tank in the afternoon

(Reebs, 1999). Ants learned to go to one of three distinct locations, at three different times of day, to find and consume honey (Schatz et al., 1994). Hummingbirds learned which of four patches of artificial flowers was reinforced with a sucrose solution at different times of the day (Tello-Ramos et al., 2015). In addition, rats were able to learn which levers provided reinforcement in morning and afternoon sessions (Carr & Wilkie, 1997b; Mistlberger, de Groot, Bossert, & Marchant, 1996). Rats have also learned a three-sessions-per-day TPL task, in which different levers provided pellets in the morning, midday and afternoon (Carr, Tan, & Wilkie, 1999).

To solve daily TPL tasks, animals rely on endogenous oscillators (Mistlberger et al., 1996). Two main oscillators have been studied in daily TPL. The first is a light-entrainable oscillator (LEO) believed to reside in the suprachiasmatic nucleus (SCN), which is located in the mammalian hypothalamus (Mistlberger et al., 1996). Oscillations of cells in the SCN become entrained to the light/dark cycle as a result of environmental cues received through the retino-hypothalamic tract, thereby regulating rhythms of behaviour (Mistlberger et al., 1996). The second oscillator, the food-entrainable oscillator (FEO), has an unknown anatomical location and entrains the body's rhythms to the timing of meals (Mistlberger et al., 1996). For rats, these meals must occur once (Bolles & deLorge, 1962) or twice (Boulos & Logothetis, 1990; Mistlberger et al., 1996; Mistlberger et al., 2012) daily at consistent times. Entrainment to mealtimes by the FEO is evident from observations of behavioural changes, specifically increases in physical activity that occur in the time preceding the meal, known as food anticipatory activity (Flôres, Bettilyon, Jia & Yamazaki, 2016; Mistlberger, 1994; Stephan, 2002).

Using these internal oscillators, rats are able to solve the temporal component of the daily TPL task, using either circadian or ordinal timing strategies. A circadian strategy allows the animal to learn when in a day a particular event occurs (Deibel & Thorpe, 2013). For example, a rat using a circadian strategy would learn that food is in Place A at Time 1 (e.g., 10:00) and in Place B at Time 2 (e.g., 15:00), whereas rats using an ordinal timing strategy learn the order, but not the specific time, of events (Deibel & Thorpe, 2013). For example, a rat using an ordinal strategy would learn that within a given day, food is first in Place A and then in Place B, but would not learn the times associated with these events. Rats could also use an interval timing strategy (controlled by a different oscillator) or a non-timing alternation strategy to solve daily TPL tasks. In an alternation strategy, rats do not use a timing strategy, but instead employ a simple pattern of alternation between the reinforced locations (Deibel & Thorpe, 2013). For example, a rat using an alternation strategy would learn that if food was in Place A the last time, then it will be in Place B this time. With an interval timing strategy, rats learn that the location of the food is dependent on the time since some external event. For example, the rat could learn that 3 hrs after colony room light onset, food is in Place A and 8 hrs after colony room light onset, food is in Place B.

To determine which strategies animals prefer while performing a TPL task, skip session probes are conducted. Skip session probes involve skipping a session (leaving the animal in the colony room) and observing the animal's behaviour during the following session. If animals are using a circadian timing strategy, then they will be accurate following both skipped morning and skipped afternoon sessions. If animals are using an ordinal strategy, then they will be incorrect following a skipped morning session but

correct following a skipped afternoon session. If animals are using an alternation strategy, then they will be incorrect following both skipped morning and skipped afternoon sessions (Carr & Wilkie, 1999; Deibel & Thorpe, 2013; Mulder, Gerkema, & Van der Zee, 2013; Mulder, Van Der Zee, Hut, & Gerkema, 2013). And finally, if rats are using an interval timing strategy, they should get both sessions correct after skipped sessions. To test for an interval timing strategy, we would have to manipulate the timing of the rat's external stimuli. The most likely of these would be the timing of colony room light onset and mealtimes. Pizzo and Crystal (2002) tested for the use of an interval timing strategy by conducting probe tests, after the rats had received constant light and no daily meal for 24 hrs. This was done to determine if rats used either colony room light onset or mealtime as the external cue in interval timing. They found that rats continued to search the correct location at the correct time of day although there was a decrement in performance (i.e., the proportion of correct responses decreased on probe trials). They argue that this suggests that both interval and circadian timing strategies were used. However, it is also possible that the performance decrement was due to the extremely disruptive nature of constant light and a skipped meal. A better probe test would have been to shift the timing of light onset and mealtime so that it resulted in a conflict as to which levers would be reinforced. In a study with pigeons, neither colony room lighting shifts nor constant dim lighting had an effect on performance (Saksida & Wilkie, 1994). However, to our knowledge similar probes have not been done with rats. Unfortunately, we were unable to do these colony room lighting probes as this requires additional testing space that we did not have available.

It appears that the timing strategy used depends on different factors, including the species (e.g., Biebach, Falk & Krebs, 1991; Carr & Wilkie, 1997b; Pizzo & Crystal, 2004; Saksida & Wilkie, 1994), the age of the subject (e.g., Mulder, Reckman, Gerkema & Van der Zee, 2015), and the specifics of the tasks (e.g., Deibel & Thorpe, 2013). Even within the same experiment, subjects use different strategies, indicating that there are individual differences in the preferred strategy (Carr & Wilkie, 1997b; Thorpe et al., 2003; Thorpe et al., 2012). It is also likely that subjects simultaneously use multiple strategies (Tello-Ramos et al., 2015). Hummingbirds learned to go to specific artificial flower patches at different times of day in order to receive a reward, but on a test day during which birds were only allowed access to the flowers during the fourth time of day and all four patches were unrewarded, no preference for either patch was observed (Tello-Ramos et al., 2015). If the birds had been using just a circadian strategy to solve the task, they would have preferred the patch that was always rewarded at the fourth time of day. If the birds had been using just an ordinal strategy, they would have preferred the patch that was rewarded first each day. Because the birds were able to learn the task, the authors concluded that aspects of both ordinal and circadian strategies combined in order to guide the birds' behaviour (Tello-Ramos et al., 2015). It would appear that there may be some redundancy built into the system so that animals can better keep track of the spatiotemporal regularity of biologically significant stimuli, such as food.

While a significant amount of research has examined how animals learn about food availability that varies reliably with time of day, there is a lack of research on how animals track periods of non-availability of food. The goal of the current study was to determine if rats are able to simultaneously keep track of periods of food availability and

non-availability. And if rats can solve these types of tasks, we wanted to determine which type of timing strategy they use (circadian, ordinal, or alternation). To answer these questions, rats were trained on a daily TPL task in which they received three sessions per day on an elevated plus maze. For one group of rats (Group All), each session had an associated reinforced arm. For example, Arm 1 was reinforced in morning sessions, Arm 2 was reinforced in midday sessions, and Arm 3 was reinforced in afternoon sessions. Based on previous research by Carr, Tan, and Wilkie (1999) it was expected that rats would learn this task. For the second group of rats (Group Partial), only two of the three sessions were associated with a reinforced arm. For example, Arm 1 was reinforced in morning sessions, Arm 2 was reinforced in afternoon sessions, and Arm 3 was never reinforced. It was expected that rats would learn at which time of day reinforcement was not given. This hypothesis was based on previous Go-No Go experiments, in which rats had two daily sessions on a T-maze or a straight runway (Means, Arolfo, Ginn, Pence, & Watson, 2000; Thorpe et al., 2003). Food was available at both ends of the T-maze, or at the end of the runway, during only one of these sessions. The rats did not need to learn the location of the food, instead they only needed to learn the time of day that the food was available. Rats had shorter latencies to reach the food location for sessions when food was available (Means, Arolfo, Ginn, Pence, & Watson, 2000; Thorpe et al., 2003). It was therefore expected that rats in Group Partial would have longer latencies on sessions that were never reinforced compared to sessions that were reinforced. It was unknown if the rats would first learn the spatial (i.e., develop a preference for the locations that provide food) or the temporal (i.e., go faster on reinforced trials) components of the task.

Method

All methodologies used in this study were carried out in accordance with the Canadian Council of Animal Care (CCAC) Guidelines.

Subjects

Sixteen male Long-Evans rats were obtained from Charles River (St. Constant, Quebec, CA) and were randomly assigned to one of two groups which differed based on reinforcement; Group Partial rats (*n*=8) were reinforced in two of the three daily sessions and Group All rats (*n*=8) were reinforced in all three daily sessions. Rats weighed an average of 304 g at the start of the experiment. All rats were singly housed in conventional clear plastic cages (45 x 25 x 21 cm) and were maintained on a 12hr:12hr light-dark cycle, with lights on at 07:00. Each cage contained corncob bedding (Necto Company, New York, NY), shredded paper (Crink-l'Nest, Kraft, The Andersons, Inc., Maumee, OH, USA) and cotton squares. Rats were given Nylabones (Nylabone Products, Neptune, NJ), wooden blocks, and plastic tubes. Rats had *ad libitum* access to water, except during shaping and experimental sessions, and were fed standard Laboratory Animal Feed (PMI Nutrition International, St. Louis, MO) on a restricted feeding diet. A weight gain of approximately 10 g per week was allowed. Mealtimes were once a day between 16:30 and 18:00.

Apparatus

Operant Box. Shaping took place in a clear Plexiglas operant box (47 x 47 x 32 cm) placed on a counter in a windowless room measuring 243 cm x 182 cm. There was a poster on one wall and a door on another wall, which could be used as visual cues. Each wall of the operant box contained: a retractable lever (Med Associates Inc., St. Alban,

Vermont, USA, model number ENV-112CM), a light, a food cup (Med Associates Inc., St. Alban, Vermont, USA, model number ENV-200R1AM), and a pellet dispenser (Med Associates Inc., St. Alban, Vermont, USA, model number ENV-203-45). The floor of the box was covered in wood chip bedding (P.W.I. Industries Inc., St-Hyacinthe, Quebec, Canada).

Elevated Plus Maze. The maze was located in a room that measured 470 cm x 288 cm and contained various visual cues located at fixed positions, including: windows, a door, two tables, shelves, and a curtain. The maze was elevated 89 cm above the floor and consisted of a start arm and three choice arms. Each choice arm was 53 cm long and 20 cm wide and contained an electrifiable grid floor over a portion of the arm, which, for the purposes of the current experiment, was deactivated. The start arm was the same length and width as the choice arms but did not have an electrifiable grid floor. At the end of each choice arm was: a lever (Coulbourn Instruments, model number E21-03), a light, a food cup (Med Associates Inc., St. Alban, Vermont, USA, model number ENV-200R1AM), and a pellet dispenser (Med Associates Inc., St. Alban, Vermont, USA, model number ENV-203-45), which were mounted on a Plexiglas wall (27 cm high). The beginning of the start arm had two retractable levers mounted on a Plexiglas wall (29 cm high), which were retracted throughout the experiment.

Procedure

Rats were initially shaped to lever press in the operant box. They then received additional shaping sessions on the elevated plus maze before beginning the TPL task.

Shaping. All rats were shaped to lever press in the operant box with only one of the four levers available, until they were consistently pressing on a variable ratio (VR) 4

schedule, meaning they received reinforcement after approximately every four presses. Rats were rewarded with 45 mg Dustless Precision Pellets (BioServ, Frenchtown, NJ). Once rats were consistently pressing on a VR4 schedule in the operant box, they were moved to the elevated plus maze and shaping continued until they were consistently pressing on a VR15 schedule, with all three levers providing reinforcement.

TPL Training. Rats were trained during three sessions a day (10:00, 12:30, and 16:00). Each session lasted 4 mins and correct responses were reinforced on a VR8 schedule. For Group All, all three of the daily sessions were rewarded, with a different lever providing reinforcement at each time. For example, Lever 1 was reinforced at 10:00, Lever 3 was reinforced at 12:30, and Lever 2 was reinforced at 16:00. This was consistent throughout the experiment and was counterbalanced among rats. For Group Partial, a different choice arm was baited for two of the three daily trials. For example, Lever 1 might have been rewarded at 10:00 and Lever 2 at 16:00. Lever 3 would have never provided reinforcement and the 12:30 session would have never been reinforced. Reinforcement locations and sessions remained the same for each rat throughout the entirety of the experiment and contingencies were counterbalanced among rats. Timing strategies were determined through the use of skip session probes, which began after the rats reached a predetermined criterion (i.e., 13 correct sessions out of 18 for Group All and 18 correct sessions out of 20 for Group Partial). After a skip session probe, a rat was required to reach four correct sessions out of five, or four correct sessions in a row, before undergoing the next skip session probe. Rats received two skip session probes at each of the three times of day, and a third if the results of the first two were inconclusive.

Results

To quantify how both groups of rats learned their respective tasks, the average number of days to criterion for each group was examined. The chance probability of success on each session was calculated to be .33 for Group All, because there were three different locations in which Group All rats were reinforced. For Group Partial, we set the chance probability of success to .5 because there were only two different locations in which Group Partial rats were reinforced, and we wanted to account for the possibility that rats would quickly learn to restrict their responding to the two levers that provided food. If we did not account for this possibility with the Group Partial rats, then a learned place preference (i.e., preferring the levers that provided food over the one that never provided food) would bias responses toward reaching criterion even if there was no learning of the time-place aspects of the task. Therefore, criterion was set at 13 correct sessions out of 18 (binomial probability = .00016) for Group All and 18 correct sessions out of 20 (binomial probability = .00020) for Group Partial, but only counted sessions where there was reinforcement. Group All rats reached the criterion of 13/18 in an average of 35.88 days (SD = 15.73) and Group Partial rats reached the criterion of 18/20 in an average of 42.38 days (SD = 21.29). Only seven of the eight Group Partial rats reached criterion. The rat that failed to reach criterion was removed from the experiment after 80 days and 88 days to criterion was used in calculations as a "best case scenario". A two-sample t-test found no differences between the groups in the days to criterion, $t(14) = -0.65$, $p = 0.526$, partial $\eta^2 = 0.029$.

To further examine acquisition of the task in both groups, the percent correct responses for first lever press across training were examined. To do this, the data were

divided into blocks of five days. This acquisition is shown in Figures 1 (Group All) and 2A (Group Partial). It appeared that for both groups by Block 4 the rats had learned the task. This was confirmed by one-sample t-tests. The percent correct first lever press scores for Group All rats were significantly greater than chance (33%) by Block 4, $t(5) = 4.273$, $p = 0.008$, partial $\eta^2 = 0.79$ (see Figure 1). The percent correct scores for Group Partial rats were significantly lower than chance (50%) at Block 1, $t(7) = -2.806$, $p = 0.026$, partial $\eta^2 = 0.53$, but improved to chance levels by the following block and were significantly greater than chance by Block 4, $t(6) = 4.250$, $p = 0.005$, partial $\eta^2 = 0.75$, suggesting that the rats in this group were choosing the correct lever at an above chance level by Block 4 (see Figure 2A). Note that the chance levels are different for the two groups, despite the presence of three levers in all cases. Because Group Partial rats quickly learn that they must choose between two options and avoid the lever that is never baited, it is more appropriate to consider chance to be 50% for this group.

For the Group Partial rats, a paired-samples t-test was conducted to determine by which block the latencies to first lever press differed significantly for the baited versus the non-baited sessions. As shown in Figure 2B, by Block 2, the rats were making a first lever press more quickly when the sessions were baited than when they were non-baited, $t(7) = 3.032$, $p = 0.019$, partial $\eta^2 = 0.57$, suggesting that they had learned the sessions in which food was unavailable.

To examine whether Group Partial rats learned to avoid the lever that was never baited, we investigated whether the incorrect first presses were on the never baited lever or on the other baited lever. Figure 2A shows the percent responses on the correct, other baited, and never baited levers across training. The rats quickly learned to press the

correct lever, and even when the first press was incorrect, it tended to be on the other baited lever as opposed to the lever that was never baited, although paired samples t-tests showed that this difference was not significant until Block 7, $t(4) = 9.798$, $p = 0.001$, partial $\eta^2 = 0.96$.

Skip session probes were conducted to determine the timing strategies used by each of the rats. Tables 1 (Group All) and 2 (Group Partial) show the outcome of these tests. As can be seen in Tables 1 and 2, rats had difficulty with the skip session probes. Only three out of the eight rats in Group All were clearly using a circadian strategy. Another two rats were correct following both Skip AM and Skip PM sessions, indicating the use of a circadian strategy. However, they were impaired following Skip MD sessions, incorrectly pressing first on the lever normally rewarded during the MD session, indicating an ordinal strategy. This suggests that perhaps these rats were using a combination of these two strategies. The other three rats did not show a clear pattern and therefore the strategy or strategies used to solve the daily TPL task could not be determined.

At least six out of the seven rats in Group Partial were using a circadian strategy. The remaining rat appeared to use a combination of circadian and ordinal timing strategies. Some of the skip session probes for the rats in Group Partial occurred immediately prior to non-reinforced sessions, which had no "correct" lever. Therefore, latencies were analyzed for the purpose of making comparisons. Table 3 shows the average latencies to first lever press for the sessions following skip session probes that occurred prior to a non-reinforced session, the average latencies to first lever press for the sessions following skip session probes that occurred prior to a reinforced session, and the

baseline latencies for each of the Group Partial rats. One set of baseline latencies was calculated by averaging the latencies for the five non-rewarded sessions just prior to the rats reaching criterion. Another set of baseline latencies was calculated by averaging the latencies for the five rewarded sessions just prior to the rats reaching criterion. Paired samples t-tests were used to analyse the first lever press data. The average latencies to first lever press in non-rewarded sessions following two skip session probes were significantly longer than the average baseline latencies in the five rewarded sessions directly preceding criterion, $t(6) = 3.270$, $p = 0.017$, partial $\eta^2 = 0.64$. The average latencies to first lever press in non-rewarded sessions following two skip session probes were not significantly different than the average baseline latencies in the five non-rewarded sessions directly preceding criterion, $t(6) = 0.870$, $p = 0.418$, partial $\eta^2 = 0.11$. This indicates that despite the rats not having a session prior to the typically non-rewarded sessions, the rats still took longer to press a lever during those sessions. This means that the rats were able to use a circadian timing strategy to determine if food would be available in a particular session. To verify that the rats were not simply slower to make a first press after any skip session probe, a paired samples t-test was used to compare the average latencies to first lever press in non-rewarded sessions following two skip session probes with the average latencies to first lever press in the rewarded sessions following all other skip session probes. These latencies were significantly different, $t(6) = 3.150$, $p = 0.020$, partial $\eta^2 = 0.62$. The rats had longer latencies to first press on non-rewarded sessions following skip session probes than on rewarded sessions following skip session probes.

Discussion

This study was designed to show whether rats could simultaneously learn the times and locations of both food availability and non-availability. Rats were clearly able to learn these discriminations. Rats in Group All were trained at three times of day and all three sessions were reinforced, whereas rats in Group Partial were also trained at three times of day but only two of these sessions were reinforced. Rats in both groups learned the task, with both groups reaching higher than chance levels of percent correct first lever press by Block 4 (see Figures 1 and 2A). As expected, rats in Group Partial quickly learned which session was never rewarded. By Block 2, the latencies to make a first lever press were significantly longer for sessions that were non-reinforced than for sessions that were reinforced (see Figure 2B). As observed in previous studies where rats performed Go-No Go tasks (Means et al., 2000; Thorpe et al., 2003), the rats learned that there was no benefit to quickly pressing a lever at times of the day that were non-reinforced. In a natural setting, it would be wise for an animal to conserve energy if the chance of exploiting a potential food source was low. Expending energy only when food availability is a strong possibility would be beneficial. The rats in Group Partial also learned to avoid the lever that was never rewarded. As seen in Figure 2A, the rats tended to press the correct lever first, but even when they did not press the correct lever, they tended to press the other rewarded lever as opposed to the lever that was never rewarded. When feeding in nature, it is beneficial for rats to focus their foraging on locations known to provide food. The rats in the current study learned the contingencies of both reinforcement and non-reinforcement.

The results of this study are comparable to those of Thorpe and Wilkie (2007), who trained rats that at one time of day a small reinforcer was available in Location A and at another time of day a large reinforcer was available in Location B. Similar to our study, rats quickly learned the time of day in which the large reinforcer was available, as evidenced by the shorter latencies compared to trials with the small reinforcer. Rats then learned the location in which the small and large reinforcer were available. It would appear that our rats also first learned the amount of food given at each time of day and then the location in which the food was available. However, in our case they learned that there were similar amounts of food at two times of day and no food at a third time. Skip session probe tests indicated that the rats in the Thorpe and Wilkie (2007) study were using a circadian timing strategy. Similarly, in our group that received different amounts of food at different times of day (i.e., Group Partial), we found that at least six out of the seven rats were using a circadian strategy, as opposed to only three out of the eight rats in Group All.

Experiment 2: Interval Time-Place Learning

In interval TPL tasks, animals are trained that the location of a food reward varies based on the time since the start of some external stimulus (Thorpe, Hallett, & Wilkie, 2007). For example, in a typical interval TPL task, rats in an operant box are trained that Lever 1 provides reinforcement on a VR schedule for the first 5 mins, followed by Lever 2 for the next 5 mins, Lever 3 for the next 5 mins, and finally Lever 4 for the last 5 mins (Carr, Tan, Thorpe & Wilkie, 2001; Thorpe et al., 2007). In these tasks, a VR schedule is used to reduce the likelihood that rats will rely on a win-stay/lose-shift strategy. That is, a VR schedule reduces the likelihood that a rat will simply press a lever until it stops

providing reinforcement and then move to the next lever. There are three pieces of evidence that suggest that rats use a timing strategy to solve such tasks, rather than a win-stay/lose-shift strategy. First, rats show evidence of anticipation. This is when responding on a lever begins just prior to that lever being rewarded (Thorpe et al., 2007). Second, rats show anticipation of depletion, which is when a rat decreases responding on a rewarded lever just prior to the end of the lever's reinforced period (Thorpe et al., 2007). Third, on probe trials in which all levers are rewarded, rats tend to go to the previously rewarded levers at approximately the correct times (Thorpe, Petrovic, & Wilkie, 2002). It is worth noting that on these probe trials, the timing of the switching between levers tends to be less precise than during the baseline sessions, indicating that a combination of strategies must be at work (Thorpe et al., 2002). There is likely a "built-in error checker" that rats use to help them determine when to switch levers (Thorpe et al., 2002). If a lever fails to provide reinforcement within a certain time frame, the rat moves to the next lever. Rats seem to attend to whether a lever is still being rewarded, in conjunction with the use of an interval timing strategy (Thorpe et al., 2002).

To solve an interval TPL task, animals use an interval timer. This is a separate clock system from the circadian pacemaker located in the suprachiasmatic nucleus, and times short durations, generally only seconds or minutes in length (Carr & Wilkie, 1998). Both rats (Carr & Wilkie, 1998) and pigeons (Wilkie, Saksida, Samson, & Lee, 1994) appear to time each lever's reinforced period individually and successively, rather than timing the entirety of the session.

This interval clock has the properties of start, stop, reset, and restart (Thorpe et al., 2002). These properties have been elucidated in interval timing tasks and specifically

interval TPL tasks, often by introducing a distractor in a probe test. According to Buhusi's time-sharing theory (Buhusi, 2012), the less salient a distractor, the smaller the effect will be on the timing of an interval. If a highly distracting event occurs during the timing of an interval, an animal will not be able to adequately distribute resources to the processing of both events and will stop timing the interval and restart timing following the withdrawal of the distracter. For example, in the Buhusi study (2012), rats who were distracted during a Fixed Interval 30 s task were only distracted by higher intensity tones, but not lower intensity tones.

With respect to interval TPL tasks, Thorpe et al. (2002) found that if rats that were previously trained on an interval TPL task were given a distractor (i.e., a piece of highly preferred food) in the middle of the session, the rats returned to the previously pressed lever following consumption of the food. This suggests that their spatial information was intact. However, the usual anticipation effects were absent, suggesting that the clock stopped during the distraction. Because of the error-checker described above, it is not possible to determine if the clock restarted or reset after the distractor. Wilkie and colleagues (1994) found that pigeons given a time-out (i.e., all key lights turned off) during an interval TPL task, acted as if their clocks had been stopped and restarted when the key lights were re-lit.

These previous studies have shown that in the event of a sufficiently salient distracter, the internal interval-timing clock appears to stop and restart/reset (Buhusi, 2012; Thorpe et al., 2002; Wilkie et al., 1994). The purpose of the present study was to determine if rats would time periods of predictable non-reinforcement or if they would stop timing during these periods. Rats were trained on an interval TPL task. Group BR5

was trained on a standard interval TPL task in which Lever 1 provided reinforcement for the first 5 mins, followed by Lever 2 for the next 5 mins, Lever 3 for the next 5 mins, and Lever 4 for the final 5 mins. Group BR4.5 was trained that each consecutive lever provided food for 4.5 mins with a 30 s non-reinforced period between each lever. Group BR4 was trained that each consecutive lever provided reinforcement for 4 mins with a 1 min non-reinforced period between each lever. See Figure 3 for a diagrammatic representation of these contingencies. It is possible that when faced with periods of non-reinforcement, rats may stop timing during these intervals. Alternatively, they may time these periods in the same manner as they time the interspersed rewarded periods. Timing may begin from the start of the session and continue throughout, or it may start and stop with each new interval. The present study aimed to clarify the question of if, and how, rats may be timing periods of food non-availability. If rats learn the spatial and temporal contingencies, regardless of the length of the non-reinforced breaks, they should show anticipation of reward, and anticipation of depletion, on the correct lever at the correct time during the sessions. Probe tests were conducted to determine whether rats were timing the intervals or using a win-stay/lose-shift strategy. While research has examined the properties of the internal clock being used by animals in the standard interval TPL task previously described, to our knowledge no research has examined the ability of animals to learn an interval TPL task in which there are scheduled breaks. We expected that the rats in the current study would learn the task, but it was unknown if they would use an interval timing strategy, or a win-stay/lose-shift strategy to do so.

Method

Subjects

Twenty-two male Long-Evans rats were obtained from Charles River (St. Constant, Quebec) and divided into three groups, Group BR5 (*n*=8), Group BR4.5 (*n*=8), and Group BR4 (*n*=6). All rats were housed in the same manner as rats in Experiment 1. Rats had *ad libitum* access to water, except during shaping and experimental sessions, and were fed standard Laboratory Animal Feed (PMI Nutrition International, St. Louis, MO) on a restricted feeding schedule. A weight gain of approximately 5 g per week was allowed and rats weighed an average of 301 g at the start of the experiment. Mealtimes were once a day between 16:30 and 18:00.

Apparatus

First Operant Box. The operant box used for shaping was the same as the operant box used in Experiment 1.

Second Operant Box. The experimental sessions took place in a second operant box, located in a different room than the first operant box. All box and room dimensions were the same as the first box and room. The second operant box had four non-retractable levers (Med Associates Inc., St. Alban, Vermont, USA, model number ENV-110M) with one mounted on each wall. Also, on each wall was a food cup, light, and pellet dispenser (see previous supplier and model information).

Procedure

Shaping. All rats were shaped to lever press in the first operant box with only one of the four levers activated, until they were consistently pressing on a VR 15 schedule.

Experiment. Rats received an average of five training sessions per week. Training sessions were conducted in the second operant box and occurred during different times of day, to ensure that the rats were not able to not use a circadian strategy to solve the task. For each session, rats were initially given a 2 min non-reinforced time-out period during which none of the levers were activated and none of the lights were illuminated. This non-reinforced period was used to allow the rats to explore the box prior to the start of the session proper. After 2 mins, all four lights were illuminated and remained on throughout the remainder of the session. Rats in the BR5 group were trained on the standard interval TPL task in which each lever provided reinforcement on a VR 8 schedule for 5 mins each, in succession. That is, rats had the initial 2 min non-reinforced period, followed by Lever 1 providing reinforcement for 5 mins, then Lever 2 for 5 mins, then Lever 3 for 5 mins, and finally Lever 4 for 5 mins. For the BR4.5 group, there was a 30 s non-reinforced period between each lever. The total duration of the session remained 22 mins. That is, rats had the initial 2 min non-reinforced period, followed by Lever 1 providing reinforcement for 4.5 mins, followed by a 30 s non-reinforced period, then Lever 2 for 4.5 mins, followed by a 30 s non-reinforced period, and so on. The rats in the BR4 group experienced a 1 min non-reinforced period between each lever. In total, each session lasted 22 mins. Figure 3 shows the order of lever reinforcement and length of reinforced and non-reinforced periods for rats in each group. Probe sessions, during which all levers were reinforced at all times (except during the initial 2 min time-out period), were conducted to determine if the rats were using an interval timing strategy or a win-stay/lose-shift strategy to solve the task. The duration of each probe session was also 22 mins.

Results

All rats had 64 sessions before receiving a probe session. Only the data from the last 20 of these sessions were analyzed as baseline data, as by this point the rats were behaving consistently. A computer program recorded lever presses and computed response rates for each lever in 30 s bins, for a total of 44 bins. Each rat's mean response rate for each lever was normalized by taking the mean response rate on a lever during a particular bin and representing it as a percentage of the maximum response rate across bins on that lever (i.e., within each rat and each lever, response rates for each bin were divided by the response rate from the bin that had the largest response rate). Group averages for each lever during each bin were found by averaging the rates of all group members and then normalizing these data in the same manner. See Figures 4, 5, and 6 for these baseline average response rate distributions and Figure 7 for the same baseline data with all groups superimposed in one graph to clearly see the differences.

A repeated measures 3 (Group) x 4 (Lever) ANOVA was conducted to determine if the groups differed in lever response rates across the session. Groups BR5, BR4.5 and BR4 did not differ significantly in terms of response rates, $F(2, 19) = 0.724$, $p = 0.498$, partial $\eta^2 = 0.071$. There was a main effect of lever, $F(3, 57) = 3.975$, $p = 0.012$, partial $\eta^2 = 0.173$. There was no interaction between group and lever, $F(6, 57) = 0.615$, $p = 0.717$, partial $\eta^2 = 0.061$. Follow-up paired samples t-tests, with alpha reduced to 0.01, showed that the main effect of lever was due to differences between response rates on Levers 1 and 4, $t(21) = -2.863$, $p = 0.009$, partial $\eta^2 = 0.28$. The difference between Levers 2 and 4 trended towards significance, $t(21) = -2.717$, $p = 0.013$, partial $\eta^2 = 0.26$, as did the

difference between Levers 1 and 3, $t(21) = -2.335$, $p = 0.030$, partial $\eta^2 = 0.21$. Levers 3 and 4 had higher rates of responding than Levers 1 and 2.

To determine if rats were anticipating food availability on each lever, response rates just prior to a lever's rewarded period were analyzed. The last 30 s bin occurring just before a lever's active period began was subtracted from the second last 30 s bin, and these scores served as a measure of anticipation (i.e., Bin 14 – Bin 13 for Lever 2, Bin 24 – Bin 23 for Lever 3, and Bin 34 – Bin 33 for Lever 4). If rats started to increase their responding on a particular lever just before the reinforcement period for the lever began, then we would expect this anticipation score to be positive. Anticipation was not analyzed for Lever 1, because the two bins prior to this lever's active period were part of the initial 2 min time-out when rats tend to explore and press all of the levers, so response rates during this time would be misleading. Figure 8 shows the levels of anticipation on each lever for each group. One rat had an extreme anticipation score (z-score > 3), so it was removed from the analyses. A repeated measures 3 (Group) x 3 (Lever) ANOVA showed no main effect of group, $F(2, 18) = .493$, $p = .619$, partial $\eta^2 = 0.052$, and no main effect of lever, $F(2, 36) = 2.515$, $p = 0.095$, partial $\eta^2 = .123$. There was no significant interaction between group and lever, $F(4, 36) = 1.366$, $p = .265$, partial $\eta^2 = .132$. Although there were no significant differences in Anticipation across lever and group, Anticipation, using a one-tailed test of the intercept, did differ from zero, $M = 2.463$, $SE = 1.206$, $t(18) = 2.042$, $p = .028$, partial $\eta^2 = .188$, which suggests that, in general, animals were anticipating the start of each lever's active period.

To determine if rats were anticipating depletion of food availability on each lever, response rates just prior to the end of a lever's rewarded period were analyzed (see Figure

9A). The last 30 s bin occurring just before a lever's active period ended was subtracted from the second last 30 s bin, and these scores served as a measure of anticipation of depletion (i.e., for BR5: Bin 14 – Bin 13 for Lever 1, Bin 24 – Bin 23 for Lever 2, and Bin 34 – Bin 33 for Lever 3; for BR4.5: Bin 13 – Bin 12 for Lever 1, Bin 23 – Bin 22 for Lever 2, and Bin 33 – Bin 32 for Lever 3; for BR4: Bin 12 – Bin 11 for Lever 1, Bin 22 – Bin 21 for Lever 2, and Bin 32 – Bin 31 for Lever 3). If rats started to decrease their responding on a particular lever just before the reinforcement period for that lever ended, then we would expect this Anticipation of Depletion score to be negative. Anticipation of Depletion was not analyzed for Lever 4 because it was the end of the session and any apparent Anticipation of Depletion might be confused with anticipation of the end of the session. A repeated measures 3 (Group) x 3 (Lever) ANOVA showed no main effect of group, $F(2, 19) = 2.170$, $p = 0.142$, partial $\eta^2 = .186$, and no main effect of lever, $F(2, 38) = 1.270$, $p = 0.292$, partial $\eta^2 = .063$. There was no significant interaction between group and lever, $F(2, 38) = 1.265$, $p = 0.301$, partial $\eta^2 = .118$. Not only were there no differences by Lever or Group, but overall Anticipation of Depletion only trended towards significance, $M = -2.341$, $SE = 1.543$, $t(19) = 1.517$, $p = .073$, partial $\eta^2 = .108$, which may mean that rats were not timing the end of food availability on each lever.

A repeated measures 3 (Group) x 3 (Lever) ANOVA was conducted to determine if anticipation of depletion was evident if the two 30 s bins occurring just before the start of the next lever's active period began were used as a measure of anticipation of depletion (i.e., Bin 14 – Bin 13 for Lever 1, Bin 24 – Bin 23 for Lever 2, and Bin 34 – Bin 33 for Lever 3). This meant that for BR4.5 and BR4 rats, some of this time was during the non-reinforced breaks between levers being activated. Anticipation of depletion was not

analyzed for Lever 4. The ANOVA showed a main effect of group, $F(2, 19) = 17.452$, $p < .0001$, partial $\eta^2 = 0.648$, and a main effect of lever, $F(2, 38) = 3.748$, $p = 0.033$, partial $\eta^2 = .165$. There was a significant interaction between group and lever $F(4, 38) = 6.873$, $p < 0.0005$, partial $\eta^2 = .420$. Simple main effects analyses demonstrated that there were no differences between the groups at Lever 1, $F(2,19) = 1.023$, $p = .378$, partial $\eta^2 = .097$, but there were differences between them at Lever 2, $F(2,19) = 12.836$, $p < .0005$, partial $\eta^2 = .575$, and Lever 3, $F(2,19) = 16.892$, $p < .0005$, partial $\eta^2 = .640$. For Lever 2, Tukey's post-hoc analyses demonstrated that the BR4 group had higher anticipation of depletion scores than the BR4.5 group, which had higher scores than the BR5 group. For Lever 3, Tukey's post-hoc tests demonstrated that the BR4 group had higher anticipation of depletion scores than both the BR4.5 and the BR5 groups, which did not differ from each other. Figure 9B shows the levels of anticipation of depletion on each lever, for each group, when the two 30 s bins occurring just before the next lever's active period began were analyzed.

To confirm that rats were using an interval timing strategy and not a win-stay/lose-shift strategy, two probe tests were conducted for each rat, the first one during Session 65 of training and the second during Session 75. During these tests, all of the levers were rewarded following the initial 2 min time-out and remained active throughout the rest of the session. The response rates were averaged across rats and both probe sessions to get group averages. These distributions were normalized using the procedure outlined above. The majority of the rats seemed to be timing, as they tended to shift responding to the correct lever at approximately the correct times. Figures 10, 11, and 12

show the average response rate distributions on each lever for each group for the probe sessions.

Discussion

The second experiment was designed to determine whether rats could learn an interval TPL task in an operant box when periods of reinforcement were interspersed with breaks in reinforcement. The rats learned the task as predicted, pressing mainly on the rewarded levers at the appropriate times, as is evident in the graphs of the baseline data (see Figures 4, 5, 6, and 7). Based on the fact that rats showed evidence of anticipation (see Figure 8), anticipation of depletion (see Figure 9B), and that they continued to move from lever to lever at approximately the correct times during probes tests (see Figures 10, 11, and 12), it appears that the rats were timing rather than relying on a win-stay/lose-shift strategy. The fact that the lever switches during the probe sessions were not as accurate as during the baseline sessions, indicates that during baseline sessions the rats were likely using a "built-in error checker" to assist in decision-making, as suggested by Thorpe and colleagues (2002).

The rats were anticipating the start of each lever's active period, as indicated by a one-tailed test of the intercept. The rats began pressing on a lever just prior to the lever becoming reinforced. In a natural setting, anticipatory action would convey an advantage to the rats, as being the first to a location with resources that are predictably available for a limited time would mean a greater chance of success in obtaining these resources. There were no differences in anticipation levels between groups, meaning that the length of the breaks in reinforcement did not impact levels of anticipation. There were also no

differences in anticipation based on lever. Regardless of a lever's physical location and order in the sequence, anticipation levels did not differ.

It appears that the rats in all groups were timing the 5 min periods. For example, if rats in the BR4 group were timing the 4 min periods, we would have expected to see anticipation of depletion at the end of each 4 min period. This was not the case. The failure to see evidence of anticipation of depletion was not likely due to the relatively small sample size. Previous studies that looked at anticipation of depletion showed a very large effect (Thorpe et al., 2007), so for our power analysis, a conservative and conventional Cohen's f = .4 was used. A sample size of 12 would have been sufficient to obtain the desired effect size, with power = .8. While there was no anticipation of depletion at the end of the reinforced periods, there was evidence of anticipation of depletion at the end of the non-reinforced breaks between levers, suggesting that the rats were timing when those periods were ending. Note that it is possible that the rats decreased responding during these periods because they were not being reinforced. There were no differences between the groups at Lever 1, perhaps because all of the rats may have shown a preference for staying at the first rewarded lever and an initial reluctance to switch levers. For Lever 2, the BR4 group had the highest anticipation of depletion scores, followed by the BR4.5 group, and finally, the BR5 group. For Lever 3, the BR4 group had higher anticipation of depletion scores than all other rats, which did not differ from each other. It may be that the longer non-reinforced periods experienced by the BR4 rats encouraged a decrease in pressing at the end of the 5 min periods. Logically then, the BR5 rats would have the lowest anticipation of depletion scores, with the BR4.5 rats somewhere in the middle of the other two groups, which appears to be the case.

Based on the combined evidence of anticipation, anticipation of depletion (at the end of the 5 min periods), and performance on the probe trials, it appears that rats in all three groups were using a timing strategy to solve the interval TPL task. Surprisingly there were no differences between the three groups in terms of anticipation or anticipation of depletion at the end of a lever's active period. The levels of anticipation of depletion at the end of a lever's 5 min period did differ based on group. The rats with the longest non-reinforced periods between levers showed higher levels of anticipation of depletion.

The rats did not appear to be timing the non-reinforced periods, as they did not stop pressing during these breaks. It is likely that the rats learned to time 5 min intervals, rather than timing the reinforced and non-reinforced periods individually. The rats would therefore press during the breaks, while still showing anticipation of reward and anticipation of depletion (at the end of the 5 min periods).

General Discussion

While it has been well established that rats are able to learn when and where biologically important events occur, not as much is known about rats' ability to learn TPL tasks in which periods of non-availability of food are interspersed with periods of food availability. In two different paradigms, daily and interval time-place learning, we found evidence to suggest that rats can in fact learn the tasks even when there are periods during which food is unavailable.

In the first experiment, rats that were only reinforced on two of the three daily sessions quickly learned which levers provided reinforcement, as evidenced by their tendency to respond more quickly on reinforced sessions compared to non-reinforced sessions. These rats also learned to avoid the lever that never provided reinforcement.

Furthermore, they were able to learn which levers provided reinforcement at which times of day. In fact, the only difference between the group with a non-reinforced session and the group with no non-reinforced sessions, was the tendency for rats with the non-reinforced session to use a circadian strategy, whereas the rats that were reinforced on all sessions tended to use a combination of a circadian and an ordinal strategy. This may be because the addition of the non-reinforced session made the task more complex. Previous research suggests that tasks with higher response costs are more likely to elicit circadian timing strategies (Mulder et al., 2013). It would appear that in a daily TPL task, rats can learn when and where food is unavailable.

In the second experiment, rats were able to learn an interval time-place task whether there were no non-reinforced periods (BR5, the control group), 30 s non-reinforced periods (BR4.5), or 60 s non-reinforced periods (BR4). In all three groups, rats restricted the majority of their responding to the correct levers at the correct times. Furthermore, the fact that all groups anticipated the next lever's active period and continued to move from lever to lever at approximately the correct time on probe trials suggests that they were using a timing strategy rather than relying on a win-stay/lose-shift strategy. Unfortunately, we were unable to determine whether the rats were timing the non-reinforced periods. There appeared to be some anticipation of depletion for the end of the reinforced periods suggesting that the rats in the BR4.5 and BR4 groups were timing the reinforced periods, however this was only trending towards significance.

If the rats timed the non-reinforced periods, we might have expected to see more anticipation on the next available lever. However, this was not the case. It is also possible that if the non-reinforced periods were longer than we would have been more likely to see

evidence that they timed them. For example, in the BR4.5 group, the timing system may not have been accurate enough to distinguish the 30 s breaks, especially given that they were trained on a VR 30 schedule. We had initially intended to have a group for which the reinforced periods were 3 mins and the non-reinforced periods were 2 mins. However, it was extremely difficult to get the rats to continue lever pressing after the first lever stopped – presumably because they extinguished responding. Future researchers may want to shape the rats to continue pressing by slowly increasing the length of the non-reinforced periods. An interesting modification to the present design would be to have the lights go out during the breaks, to make the breaks more salient. It is possible that more salient breaks would have the effect of stopping and restarting the rats' internal clocks, thereby preventing learning, or at least making it more difficult. Alternatively, under conditions of increased break saliency (with either longer breaks or lights out during breaks, or both), learning may still occur, but perhaps the rats would rely on a different strategy to solve the task, such as the win-stay/lose-shift strategy.

As shown in both the daily and interval TPL tasks, rats can learn when and where food rewards will be provided, and this knowledge then guides their behaviour. Additionally, energy conservation occurs during times when no food is available, as can be seen by longer latencies to first press for the rats in Experiment 1 during non-rewarded sessions.

From the information gleaned from the two present experiments, it can be concluded that the introduction of breaks in food availability does not prevent rats from using timing strategies and solving daily and interval TPL tasks. Indeed, such breaks may make the tasks more difficult and encourage the use of more sophisticated strategies,

despite the increased neural resources that must be allotted to such strategies. If the breaks served as distractions for the rats, they were insufficiently salient to cause the rats difficulty in solving the tasks. This was evident in both the daily TPL experiment, in which some of the rats experienced one non-reinforced session per day, and the interval TPL experiment, in which some of the rats experienced non-reinforced breaks between each lever's reinforced period. This research certainly adds support to the assertion that the biological need for food is a priority that cannot be ignored.

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Table 1

First lever press choices for the sessions following the AM skip session probes, midday (MD) skip session probes, and PM skip session probes, and the timing strategy used by each rat in Group All.

Rat	AM Skips (Tested at MD)	MD Skips (Tested at PM)	PM Skips (Tested at AM)	Strategy
1	MD*, MD*	MD, MD	AM*, AM*	Circadian / Ordinal
2	AM, PM	PM*, MD, MD	PM, PM	Unknown
3	MD*, MD*	PM*, PM*	AM*, AM*	Circadian
4	PM, PM	PM*, PM*	AM*	Unknown
5	MD*, MD*	MD, PM*, MD	MD, AM*, AM*	Circadian / Ordinal
6	MD*, MD*	PM*, PM*	AM*, PM, AM*	Circadian
7	MD*, PM, MD*	PM*, PM*	AM*, MD, AM*	Circadian
8	MD*, MD*	PM*, MD, MD	MD, PM	Unknown

Note. Asterisks indicate that the first press was correct. AM, MD, and PM refer to the levers that the rats chose following the skipped session. Rat 4 only had one PM skip due to time constraints.

Table 2

First lever press choices for the sessions following the AM skip session probes, midday (MD) skip session probes, and PM skip session probes, and the timing strategy used by each rat in Group Partial.

Rat	AM Skips (Tested at MD)	MD Skips (Tested at PM)	PM Skips (Tested at AM)	Strategy
9 (MD)	AM, AM	PM*, PM*	AM*, AM*	Circadian
10 (MD)	PM, PM	PM*, NB, PM*	AM*, AM*	Circadian
12 (PM)	MD*, AM, MD*	?, ?, MD	AM*, ?, AM*	Circadian
13 (PM)	MD*, MD*	MD, MD	AM*, AM*	Circadian
14 (AM)	MD*, MD*	MD, MD	-, -	Circadian / Ordinal
15 (PM)	MD*, MD*	MD, -, -	AM*, AM*	Circadian
16 (AM)	MD*, MD*	PM*, PM*	NB, -, -	Circadian

Note. AM, MD, and PM in the first column refer to the session that was non-rewarded for that specific rat. For example, Rat 9 was never rewarded on the midday session. AM, MD, and PM in all other columns refer to the levers that the rats chose following the skipped session. Asterisks indicate that the first press was correct. Choices made during non-rewarded sessions that followed skip session probes are in shaded cells. For example, for Rat 9 the MD sessions were never rewarded, therefore following AM skip session probes there was no "correct" choice. NB indicates the rat pressed the Never Baited lever first. A dash means that the rat did not press during the session. Question marks are used to indicate missing data due to a computer glitch which resulted in

some of the data for one of the rats not recording. Rat 11 is excluded from the table as this rat did not reach criterion and did not receive skip session probes.

Table 3

Average baseline latencies to first lever press for AM, MD, and PM sessions, and average latencies for sessions that followed AM, MD, and PM skip session probes, as well as the timing strategy used, for each of the Group Partial rats.

Rat	AM Latency	MD Latency	PM Latency	AM Latency (following Skip PM)	MD Latency (following Skip AM)	PM Latency (following Skip MD)	Strategy
9 (MD)	34.8	34.3	14.7	58.3	17.3	31.2	Unkn.
10 (MD)	65.1	97.1	39.2	16.8	63.9	40.5	Circ.
12 (PM)	34.7	38.6	71.9	?	113.2	219.6[a]	Circ.
13 (PM)	41.5	33.6	216.5	32.9	40.9	86.1	Circ.
14 (AM)	192.1	52.5	28.9	241.0	151.0	48.5	Circ. / Ord.
15 (PM)	20.5	7.6	74.1	17.0	6.1	204.3[b]	Circ.
16 (AM)	177.7	15.5	11.5	187.1[b]	25.1	15.9	Circ.

Note. AM, MD, and PM in the first column refer to the session that was non-rewarded for that specific rat. For example, Rat 9 was never rewarded on the midday session. Latencies during non-rewarded sessions are in shaded cells. Rat 11 is not included in the table as this rat did not reach criterion and did not have skip session probes. In sessions where the rat did not press, 241 s was used as the latency for the purposes of

calculations. [a] This number represents the latency for the session following only one skip session probe. Due to a computer problem, the latencies for sessions following two previous skip session probes are unavailable. [b] Unlike the other averages, these are based on three latencies, as a third skip session probe was conducted to break a tie (i.e. in the session following one of the skip session probes, the rat pressed a lever and in the other, the rat did not press a lever). The question mark indicates that the latencies were unavailable due to a computer problem.

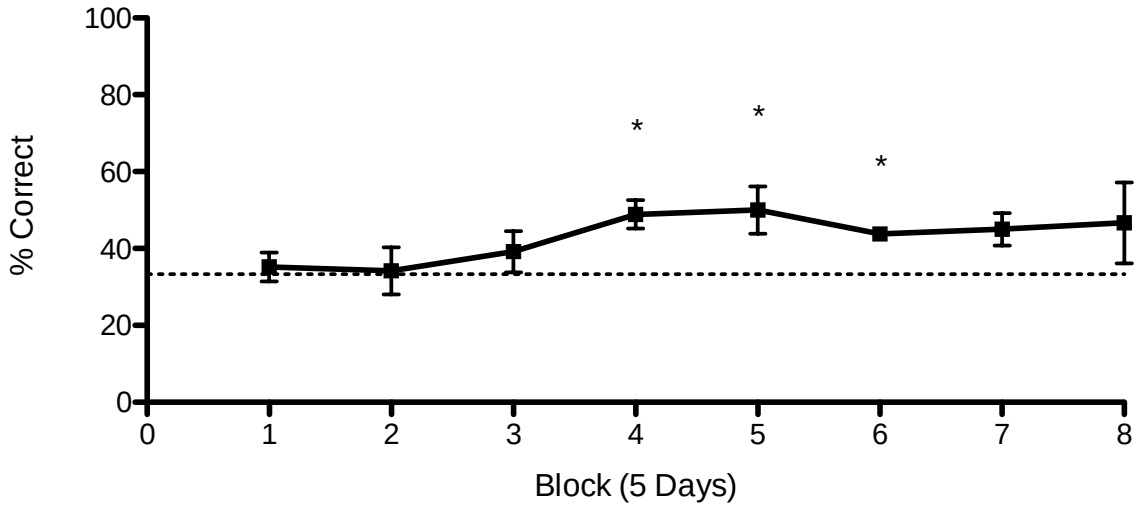


Figure 1. Percent correct first presses by block for Group All rats. The dashed line represents chance (33%). The error bars represent standard errors of the means (SEM). The asterisks indicate that for those blocks the percent correct was significantly different than chance. Once rats reached criterion and were receiving skip session probes they were no longer included in this graph. Therefore, by Block 4, only six rats were included in the average, and by Block 6, only four rats were included.

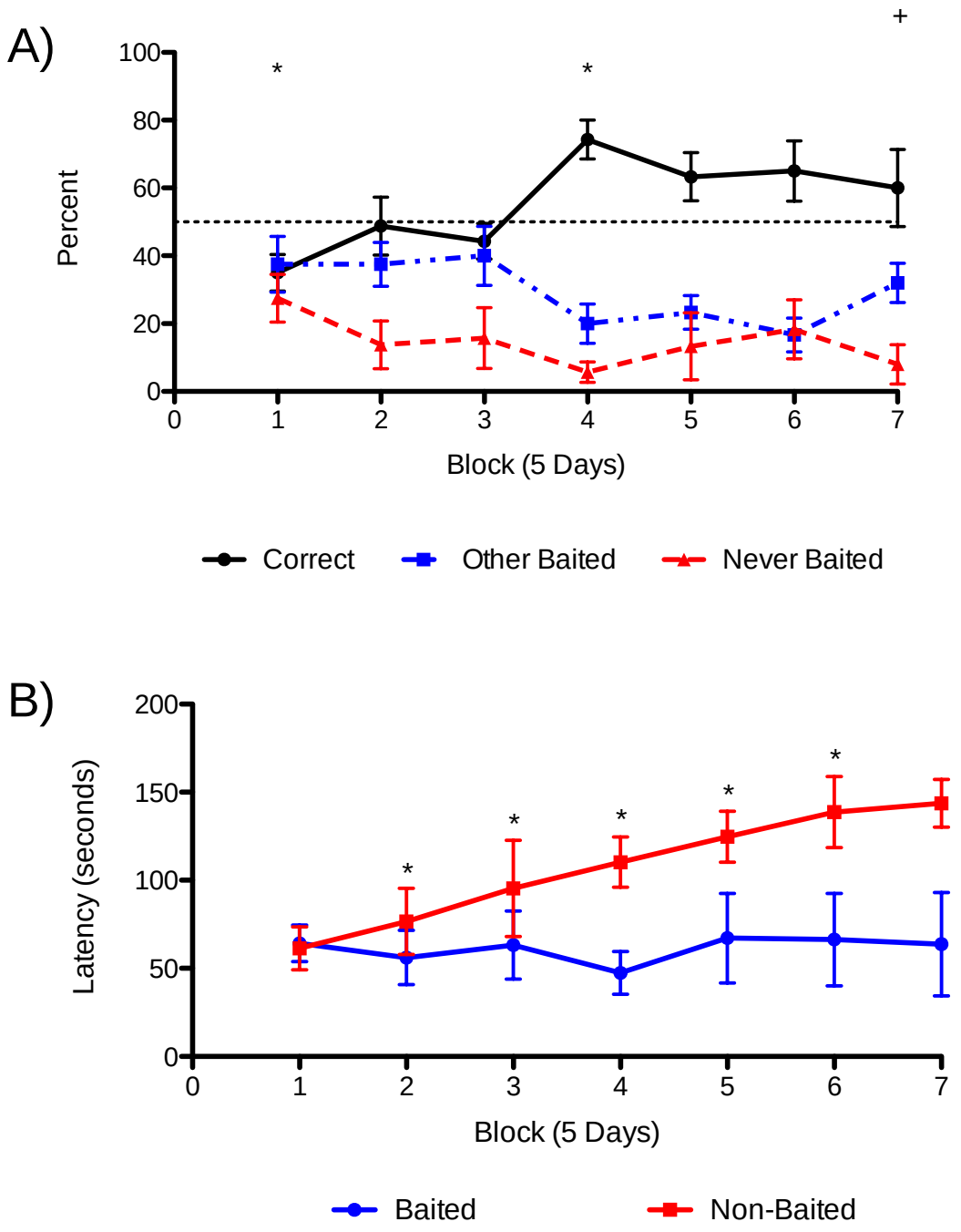
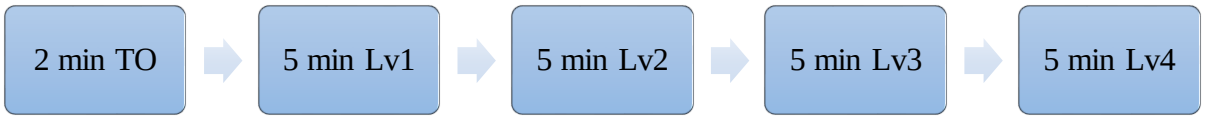


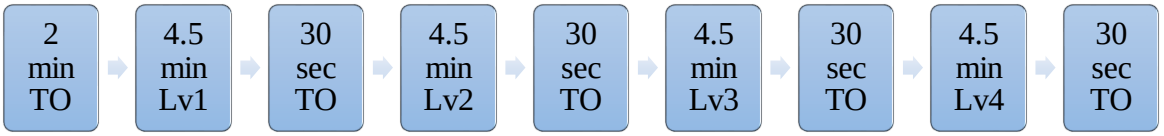
Figure 2. A) Percentage of first presses on the Correct, Other Baited, and Never Baited levers by block for Group Partial rats. The dashed line represents the 50% chance level. The error bars represent standard errors of the means (SEM). The + indicates that for that

block there was a significant difference between Other Baited and Never Baited. The percent correct was significantly lower than chance at Block 1 and significantly greater than chance by Block 4 (indicated by an *). Once rats reached criterion and were receiving skip session probes they were no longer included in this graph. Therefore, by Block 3, only seven rats were included in the average, by Block 5, only six rats were included, by Block 7, only five rats were included, and by Block 8 there were fewer than four rats included (so the average for Block 8 was not included in the graph). *B*) Mean latencies by block for baited and non-baited sessions for Group Partial rats. The error bars represent standard errors of the means (SEM). The * indicate significant differences between baited and non-baited arms.

(A) BR5



(B) BR4.5



(C) BR4

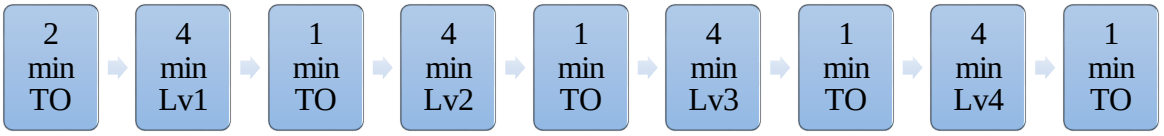


Figure 3. Order of lever reinforcement and length of reinforced and non-reinforced periods for rats in (A) Group BR5, (B) Group BR4.5 and (C) Group BR4. Note that Lv refers to lever and TO refers to time-out.

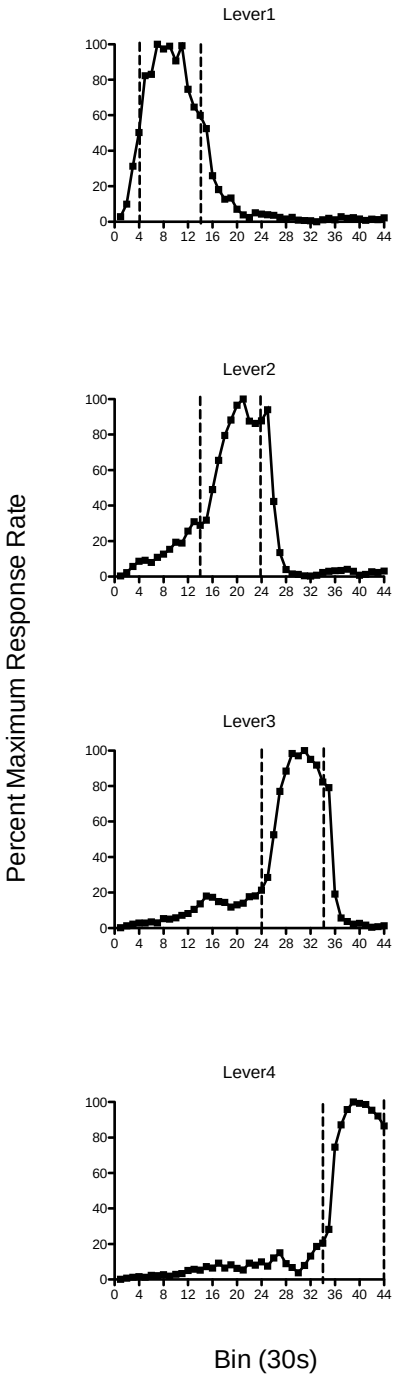


Figure 4. Baseline average response rate distributions for the last 20 sessions of training prior to the first skip session probe, averaged across all BR5 rats. Response rates are

normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. Each lever's period of reinforcement is represented by the area bound by the two dashed lines.

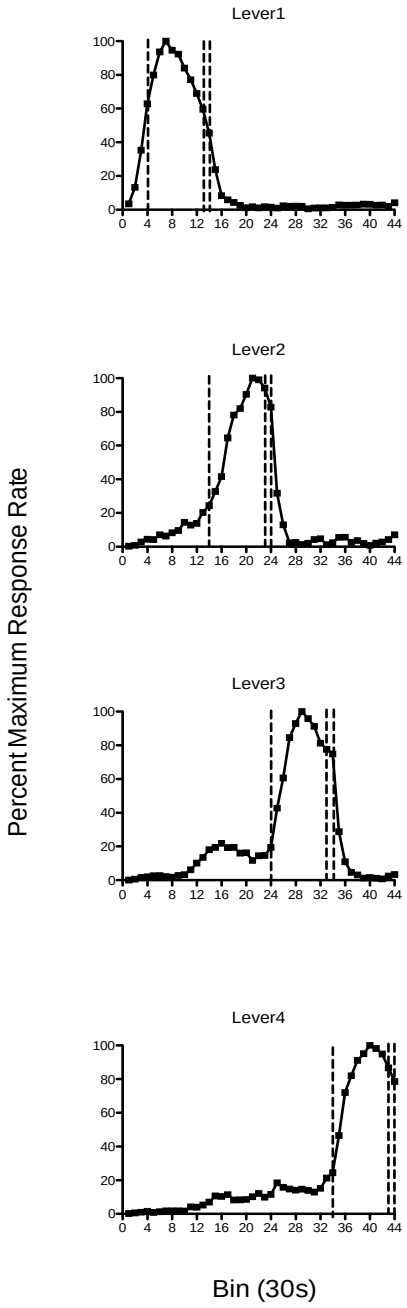


Figure 5. Baseline average response rate distributions for the last 20 sessions of training prior to the first skip session probe, averaged across all BR4.5 rats. Response rates are normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. Each lever's period of reinforcement is represented by the area bound by the first two dashed

lines, and the non-reinforced period is represented by the area bound by the last two dashed lines.

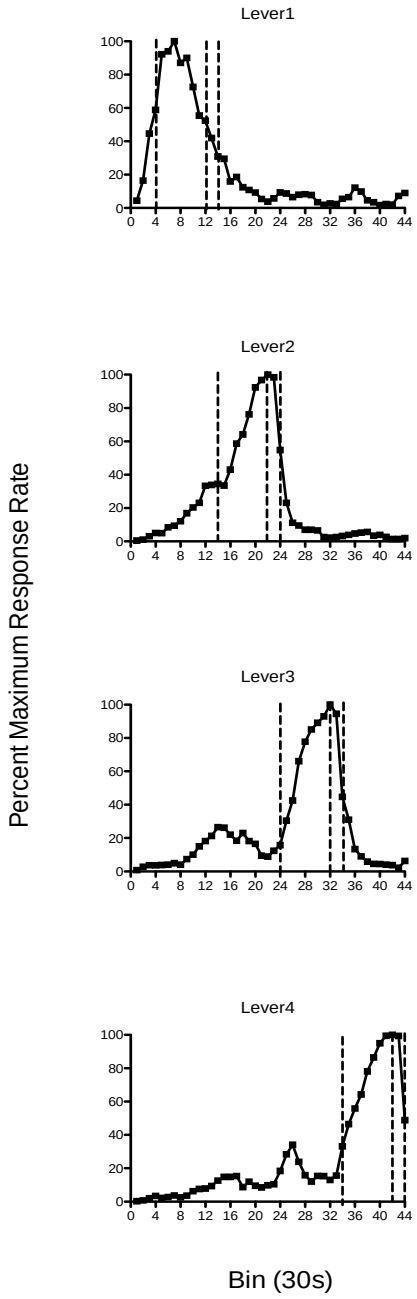


Figure 6. Baseline average response rate distributions for the last 20 sessions of training prior to the first skip session probe, averaged across all BR4 rats. Response rates are normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. Each lever's period of reinforcement is represented by the area bound by the first two dashed

lines, and the non-reinforced period is represented by the area bound by the last two dashed lines.

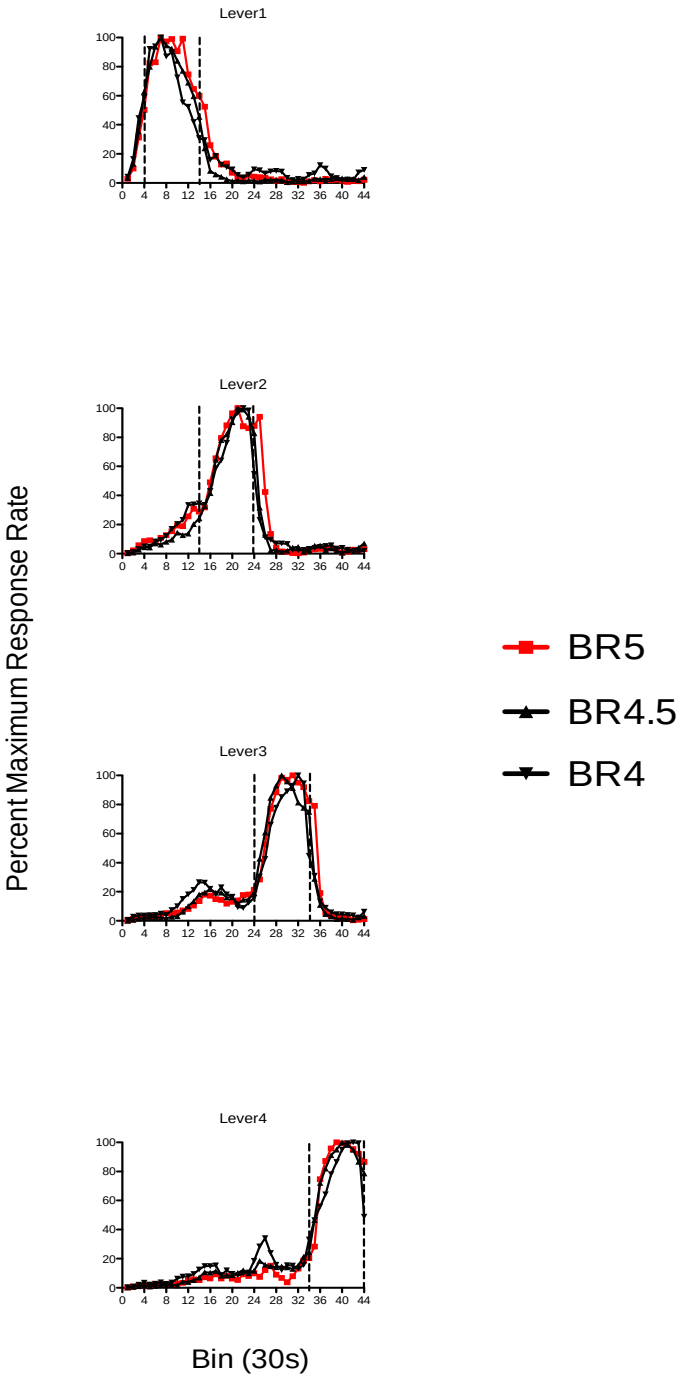


Figure 7. Group baseline average response rate distributions for the last 20 sessions of training prior to the first skip session probes. Response rates are normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. The distribution for BR5 is in red,

for easier comparison. The areas bound by the dashed lines represent the reinforced periods for BR5.

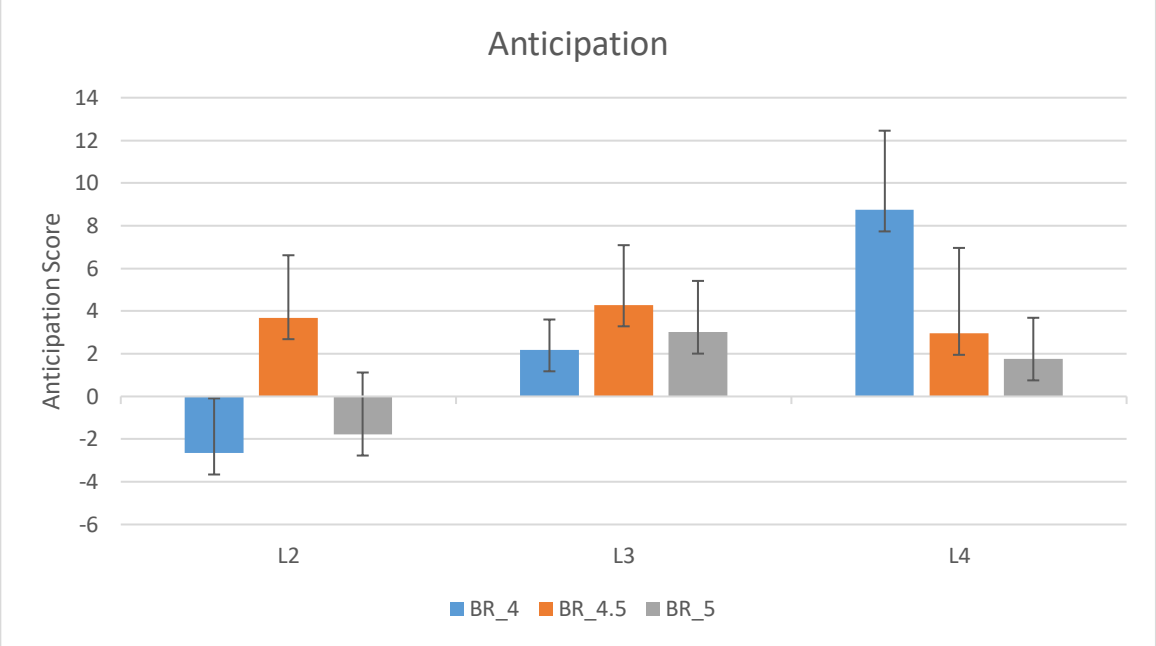
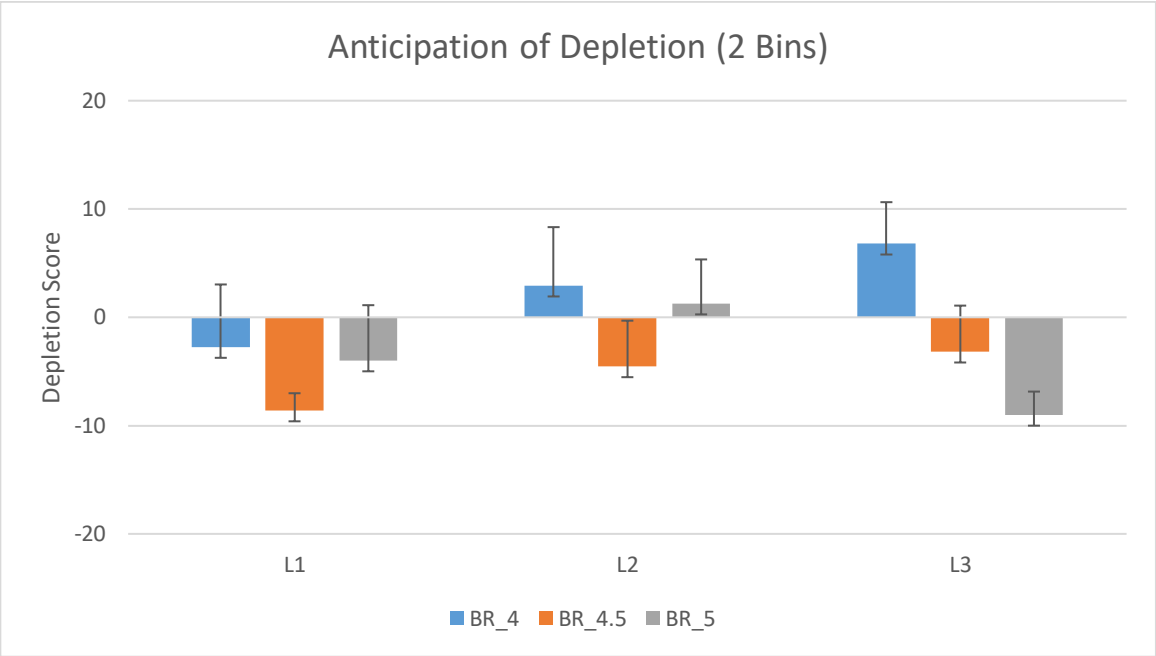


Figure 8. Each group's response rates (the last 30 s bin occurring just before a lever's active period began was subtracted from the second last 30 s bin, and these scores served as a measure of anticipation) (i.e., Anticipation Scores). The error bars represent the standard errors of the means (SEM).

A)


Anticipation of Depletion (2 Bins)
20
10
0
-10
-20
Depletion Score
L1
L2
L3
BR_4
BR_4.5
BR_5

B)

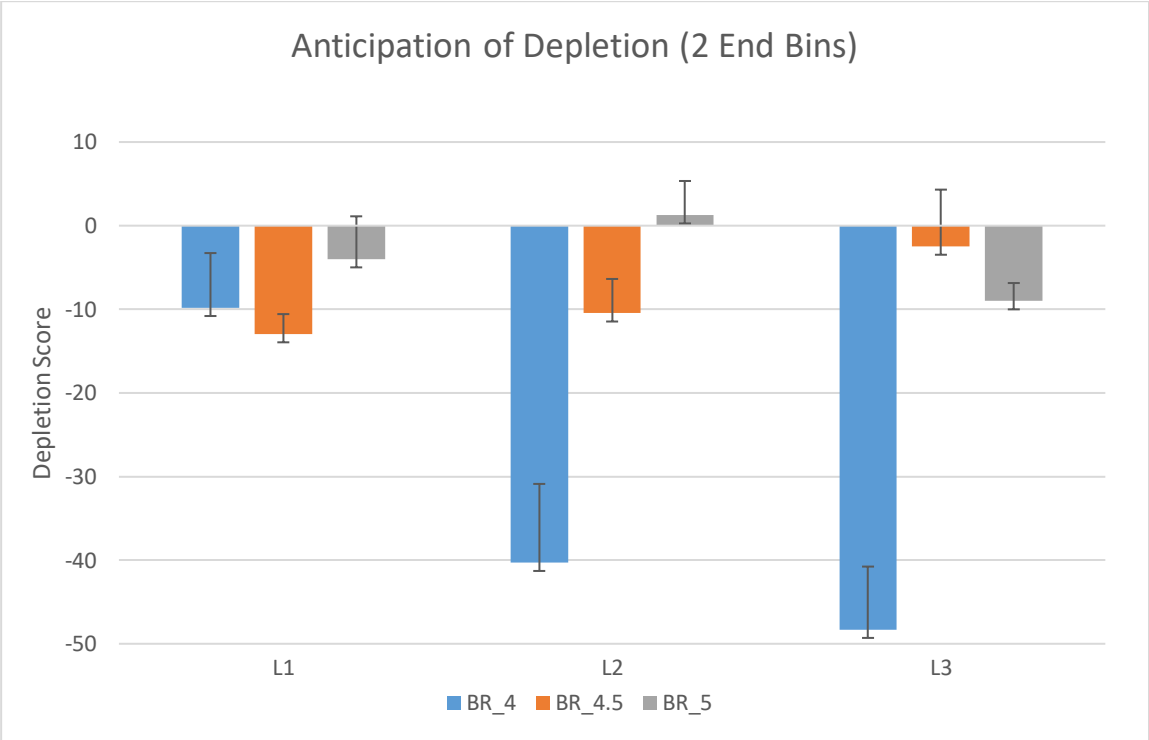

Anticipation of Depletion (2 End Bins)
10
0
-10
-20
-30
-40
-50
Depletion Score
L1
L2
L3
BR_4
BR_4.5
BR_5

Figure 9. Each group's response rates during (A) the two 30 s bins occurring just before a lever's active period ended (the last 30 s bin occurring just before a lever's active period ended was subtracted from the second last 30 s bin, and these scores served as a measure of anticipation of depletion, i.e., Depletion Scores), and (B) the two 30 s bins occurring just before the start of the next lever's active period (the last 30 s bin occurring just before the start of the next lever's active period was subtracted from the second last 30 s bin, and these scores served as a measure of anticipation of depletion, i.e., Depletion Scores). The error bars represent the standard errors of the means (SEM).

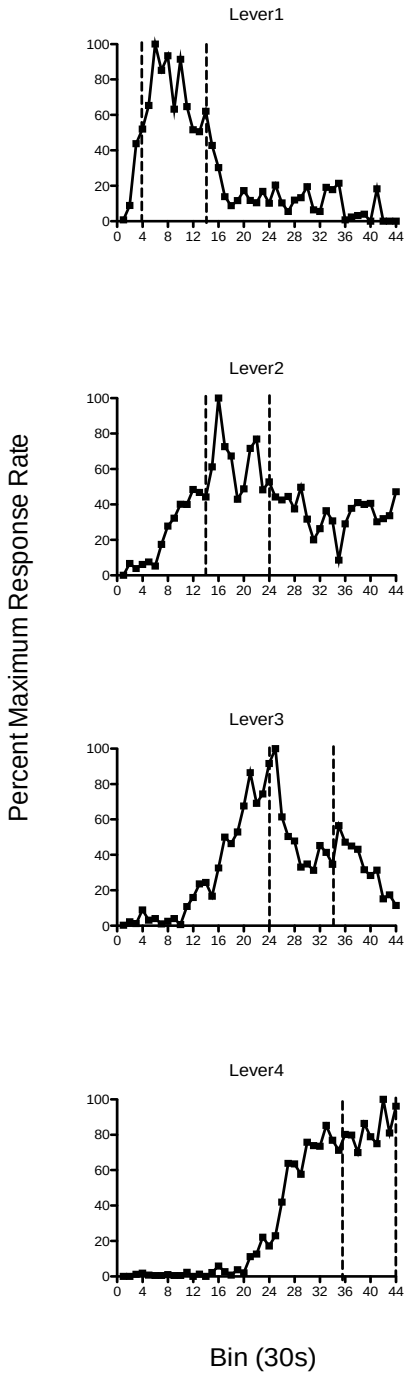


Figure 10. Response rate distributions averaged across all Group BR5 rats on two probe sessions. Response rates are normalized and averaged for each lever for each 30 s bin, for

a total of 44 bins. Each lever's former period of reinforcement is represented by the area bound by the two dashed lines.

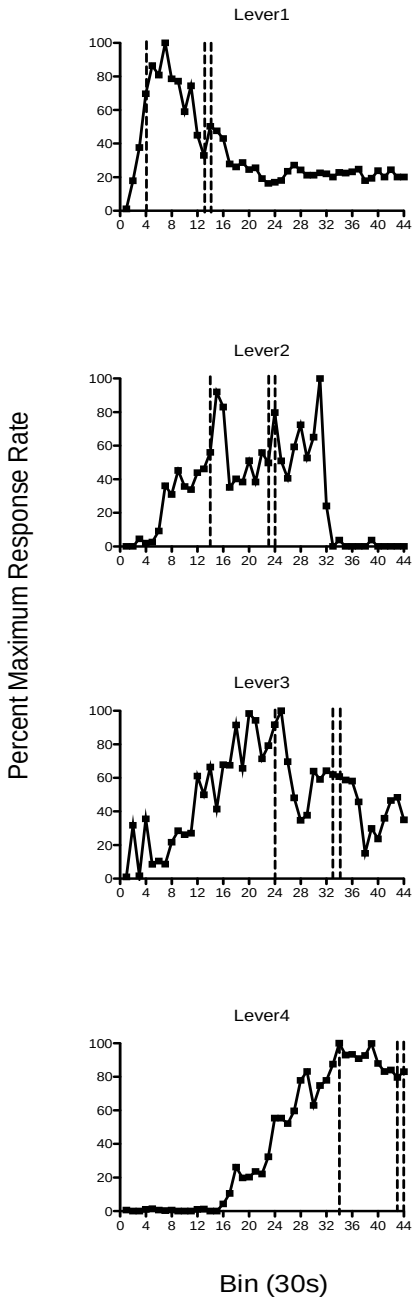


Figure 11. Response rate distributions averaged across all Group BR4.5 rats on two probe sessions. Response rates are normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. Each lever's former period of reinforcement is represented by the area

bound by the first two dashed lines, and the former non-reinforced period is represented by the area bound by the last two dashed lines.

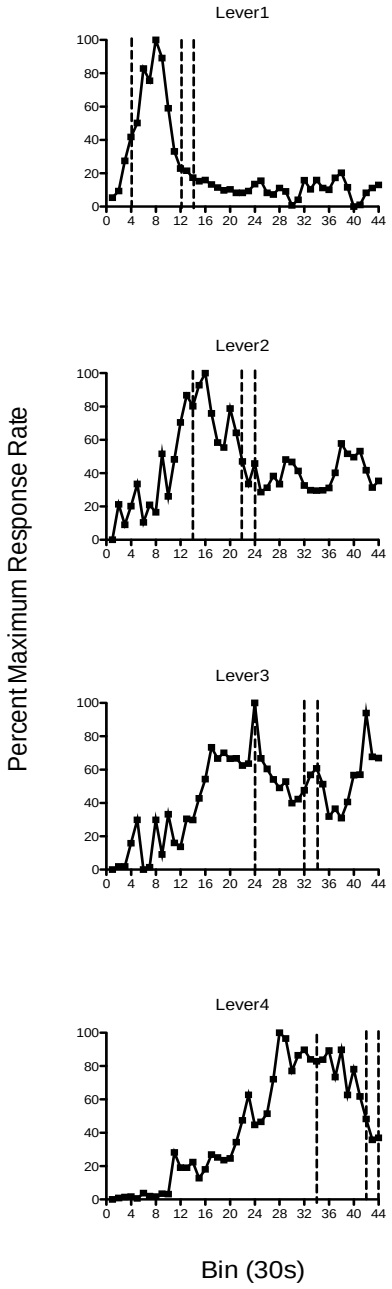


Figure 12. Response rate distributions averaged across all Group BR4 rats on two probe sessions. Response rates are normalized and averaged for each lever for each 30 s bin, for a total of 44 bins. Each lever's former period of reinforcement is represented by the area bound by the first two dashed lines, and the former non-reinforced period is represented

by the area bound by the last two dashed lines.